transport characteristics, both for the salivary glands and other epithelial tissues. This role for aquaporins has been called, by some authors, the 'simple permeability hypothesis' (SPH). Aquaporin knockout studies, in which the effects of targeted knockouts of aquaporins are considered, are most often interpreted as supporting the SPH; however, this interpretation has been challenged by some researchers, who, on the contrary, view knockout studies as providing evidence against the SPH. We consider a mathematical model for salivary secretion based on a SPH-type mechanism, and consider whether a model of this type is sufficient to account for the key features observed in aquaporin knockout studies. We conclude, contrary to the recent objections raised, that a model of this type does appear to able to account for these features. In addition, some model features appear generalisable in such a way as to account for other patterns in knockout studies - in particular the lesser/greater effect of knockouts on the transport rates of epithelial systems that transport at a lower/higher overall rate.

3309-Pos Board B170

Fusion Dependent Activation of Vesicular $P2X_4$ Receptors Leads to a Volume Increase in Alveolar Type II Epithelial Cells - Coupling Secretion and Lung Fluid Homeostasis?

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Regulated fluid transport in the lung is a necessity for maintenance of a healthy lung environment. However, the role of the alveolar epithelium, consisting of alveolar type I and II (ATII) cells, remains largely undetermined. While the ATII cell is mainly known for secretion of lung surfactant via exocytosis of lamellar bodies (LBs), it is also thought that it may assist in fluid homeostasis as well, both critical for alveolar function. Directed ion transport across the ATII cell will result in secretion or absorption of fluid into or from the alveolar hypophase, respectively. We have recently described that exocytic fusion of LBs with the plasma membrane (PM) results in a transient, non-selective, inward-rectifying, cation current across $P2X_4$ purinergic receptors located on the membranes of fused LBs *(PNAS 2011, 108(35):14503-8)*. This leads us to propose that regulation of LB exocytosis also modulates fluid transport across the alveolar epithelium via this ''fusion-activated'' cation influx. Experiments combining fluorescence and atomic force microscopy (AFM) confirmed that exocytosis of LBs leads to an instant increase in ATII cell volume that is regulated within mins. Following stimulation of ATII cells with ATP, the height of the ATII cells increased by 30% in cells when LBs fused with the PM after stimulation, but slightly decreased in cell without fusing LBs. These data indicate a link between LB fusion (and hence surfactant secretion) and regulation of fluid transport.

Motions of the Cell Surface Molecules

3310-Pos Board B171

Diffusion of DiI, Fast DiO, and TopFluor-PC in the Outer Membrane of Live E. Coli

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While there have been many studies on the diffusion of membrane lipids in eukaryotic cells, which have given insight into the structure and organization of these membranes, little is known to date of their mobility in bacterial membranes, specifically the Gram negative bacteria, *Escherichia coli*. The *E. coli* outer envelope consists of inner and outer lipid membranes that are separated by a periplasmic space containing the cell wall. The outer membrane is unique in that it is thinner than mammalian plasma membranes and consists of a phospholipid inner leaflet with a predominantly lipopolysaccharide (LPS) outer leaflet.

Here we look at the diffusion of the fluorescent lipid analogs DiI, FAST DiO, and TopFluor-PC in the outer membrane of live *E. coli* cells using single molecule imaging/tracking techniques. Lipid analog dynamics are compared in several cells - *E. coli* with no O antigen, *E. coli* with no core oligosaccharides, and a mammalian cell line – at several sampling rates. Lipid analogs deviate from free diffusion at time scales smaller than 30 fps for FAST DiO and smaller than 260 fps for TopFluor-PC. The diffusion of lipid analogs differs according to the charge of the headgroup and fatty acid composition. These differences give evidence for predictions that LPS plays a role in membrane structure and organization and may imply a lipid-based compartmentalized structure in *E. coli*.

3311-Pos Board B172

Extracellular, Membrane and Intracellular Proteins that Alter Integrin Cell Membrane Diffusion and Clustering

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Integrins are ubiquitous membrane proteins that are involved in cell adhesion and signaling across the cell membrane. We use a combination of fluorescence microscopy and methods to modulate the concentration of a single cellular component to measure its role in altering αPS2CβPS integrin diffusion or clustering. Our work provides vital information on the molecular mechanism of integrin function through altered dynamics and membrane organization. Clustering is measured using a noninvasive fluorescence resonance energy transfer assay that does not require attaching fluorescent tags to the integrin and sub-diffraction stimulated emission depletion imaging. Diffusion is measured using a fluorescent protein labeled integrin and fluorescence recovery after photobleaching. The concentration of cellular components is modulated using RNA interference, cholesterol extraction with cyclodextrin, or glass slides coated with varying concentrations of extracellular proteins. Among our interesting findings we have determined that cholesterol depletion decreases integrin clustering but results in more constrained (i.e., less Brownian) integrin diffusion. Reducing the concentrations of insulin receptor or notch membrane proteins decreases integrin clustering; and reduced concentrations of these, as well as several other membrane proteins, results in less constrained integrin diffusion. The role of cytoplasmic proteins in altering integrin clustering depends on the concentration of extracellular ligand. Reducing the concentration of certain cytoplasmic proteins such as talin and focal adhesion kinase results in less constrained integrin diffusion, while a subset including actin and vinculin increases constraints to integrin diffusion. The role of extracellular, membrane, and intracellular proteins or small molecules in altering integrin clustering or diffusion is different for integrin mutants with altered ligand affinity compared to wild-type integrin. We hypothesize that altered partitioning into membrane nanodomains is the main mechanism for altered integrin clustering and diffusion at altered cytoplasmic, membrane or extracellular protein concentrations.

3312-Pos Board B173

Alternating Two Different Diffusion States of Min Proteins on the Lipid Bilayer Revealed by Single Molecule Tracking

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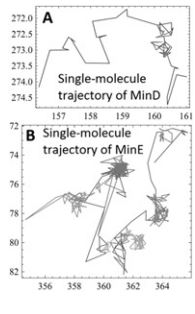
The bacterial proteins of Min family play key roles in mid-cell localization of cell division septum. MinD-ATP accumulates on the cell membrane and MinE regulates membrane release of MinD by triggering ATP hydrolysis. This results in the oscillation of MinD between cell poles generating time-average MinD concentration minimum at the mid-cell. MinC, a septum formation inhibitor, co-localize with MinD to ensure mid-cell septum formation. In vitro, the Min proteins display interconverting modes of dynamic pattern formation including propagating waves, spatially near-uniform oscillations of surface concentration, propagating filament-like structures, and mobile amoeba-like structures surrounded by MinE rings similar to the MinE ring in vivo (V Ivanov and K Mizuuchi, PNAS 107, 8071 (2010)).



We tracked single-molecules of MinD and MinE that constitute the wave pattern and found two different diffusion states: free-diffusing and trapped (Fig, Free trajectory is blue; trapped in different colors). The fraction of the trapped state of MinD molecules increases near the wave tail where MinE concentration is high and a large fraction of MinE is also in the trapped state. MinE molecules in trapped state jump between different trap locations.

3313-Pos Board B174

Clathrin-Mediated Endocytosis Introduces a Nonergodic Diffusion Process in the Plasma Membrane

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Tracking individual potassium channels in the plasma membrane reveals complex dynamics involving anomalous diffusion. Theoretical models show that